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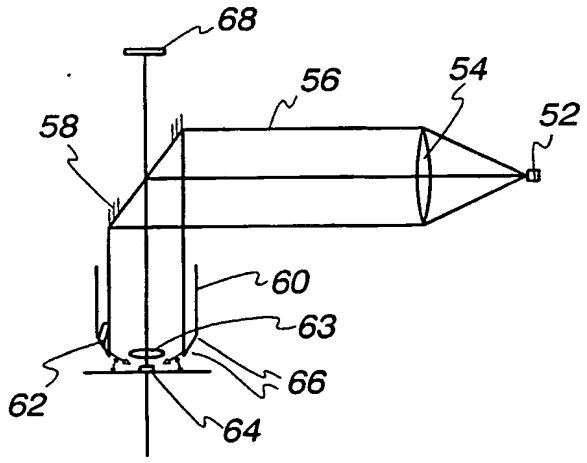
(54) Title: FLUORESCENCE MICROSCOPY METHODS AND DEVICES USING LIGHT EMISSION DIODES



(57) Abstract: A device for illuminating a sample (64) undergoing microscopic examination is provided, the device comprising a fluorescing radiation detector (68) and a light emitting diode (52) juxtaposed to the sample so as to irradiate the sample and prevent the detecting means from detecting non-fluorescing radiation from the sample.

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FLUORESCENCE MICROSCOPY METHODS AND DEVICES USING LIGHT EMISSION DIODES

CONTRACTUAL ORIGIN OF THE INVENTION

The United States Government has rights in this invention under Contract No. W-31-109-ENG-38 between the U.S. Department of Energy and the University of Chicago representing Argonne National Laboratory.

BACKGROUND OF THE INVENTION

1. Field of the Invention.

The present invention relates to a device and a method for illuminating samples in microscopy, and more particularly, the present invention relates to a device and a method for incorporating light emitting diodes in fluorescence microscopy procedures.

2. Background of the Invention

Fluorescence microscopy has many uses related to detection of target molecules. Target molecules when illuminated with excitation light may either fluoresce spontaneously or they may fluoresce after a tag is attached thereto. In the latter situation, a fluorescence tag is attached onto a moiety which itself is complimentary to the topography or chemistry of the target molecule. When the moiety finds this target, the fluorescent tag attached to the moiety indicates the existence of the duplex thus formed. One can then analyze the topography or

structure of the molecules or their relative abundance.

At present, two light sources are used for fluorescent microscopy, namely lasers and high power lamps (such as tungsten-, mercury-, and xenon-lamps). Lasers are employed mainly with scanning devices to provide a high power output in a narrow spectral region on a small surface. Lamps provide powerful illumination of large fields and also provide different wavelength capabilities. There are draw backs to both types of light sources. These drawbacks are discussed below.

Present microscope designs used in carrying out routine detection of fluorescent molecules incorporate epi-fluorescence microscopy. Epi-fluorescence microscopy most frequently employs high power tungsten or arc lamps in combination with light filters to excite the fluorescence in the sample. "Epi-" is a reference to the so-called epi-illumination scheme wherein the same objective lens is used for both illuminating the sample and collecting the fluorescence. These systems require the use of high-powered mercury or xenon lamps to ensure that after narrow-band filtering (monochro-matization), there is still enough light to provide a measurable signal at the detector. However, these sources are inefficient in that they produce a large amount of heat and light in excess of that required to illuminate the sample. Also, the large power and size requirements of these lamps makes systems incorporating them bulky. So, portability is a problem. Costs for these systems range from $12,000 to $100,000.

Lasers have been utilized in fluorescence microscopy processes. Elaborate confocal microscopy methods (i.e., confocal laser scanners) and devices exist (e.g. U.S. Patent Nos. 5,631,734 and 5,578,832) whereby sharply focused laser beams scan a sample point by point. Lasers emit light in a narrow spectral region, and as such, obviate the need for polarization filters. However, when fluorochromes are used which excite at different wave lengths, different lasers must be employed, leading to higher costs. Also, lasers do not provide enough power to illuminate the whole microscope field.

Confocal laser systems rely on complex, and therefore expensive, electronics (e.g., photomultiplier detectors and digitizing devices) to reconstruct the image as a whole. Indeed, confocal laser scanning systems cost from $500,000 to $1 million. Unlike epi-fluorescence microscopes, confocal configurations do not record the fluorescing image in parallel (i.e., all points at once).

An exemplary state of the art confocal configuration is depicted in FIG. 1 as numeral 10. Briefly, a stationary laser light source 12 supplies a light beam 14 for illuminating an objective 16. The beam is required to traverse a complex mirror- and piezo quartz-configuration 20 before arriving at the objective.

To facilitate detection of the fluorescence emanating from the now illuminated objective, the fluorescence energy must traverse through the dichroic mirror 20 to reach a detector 22 such as a photo multiplier tube. In an effort to eliminate the possibility of reflected light being detected by the detector (as opposed to just the desired fluorescent energy) a confocal pinhole 24 is juxtaposed intermediate to the objective and the detector. A signal processing unit 26 delivers the fluorescence signal to a computer 28 for processing. This configuration requires that the sample be able to move with respect to the laser (or, conversely, that the laser be able to move with respect to the sample).

The drawbacks of the above system are as follows: (a) the necessity of high precision mechanical means to move either sample or laser; (b) the impossibility of direct visual observation of the sample as the image must be processed by a computer using specially programmed software; (c) analysis takes considerable time because the sample is analyzed point by point; and (d) the instrument operator must be familiar with the fundamental features of the software.

In light of the foregoing, confocal methods are too expensive for routine fluoroscopic detection procedures.

A need exists in the art for an efficient fluorescence detection device and method which utilizes conventional microscope configurations without the need for complex signal processing units or lasers. The device and method should eliminate the energy inefficiencies and sample-desiccating heat associated with high power sources such as tungsten or arc lamps. The device and method also should utilize inexpensive light sources which are easily replaced and substituted.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a device and method for use in fluorescence microscopy that overcomes many of the disadvantages of the prior art.

Another object of the present invention is to provide an efficient fluorescence microscopy device. A feature of the invention is the incorporation of interchangeable

light emission diodes (LEDs) in the device. An advantage of the invention is enabling the device to provide fluorescence data at different excitation wavelengths. Another advantage is that the diodes can be adapted for use on a typical microscope, thereby conferring portability heretofore not seen in fluorescence microscopy systems, and at minimal cost.

Still another object of the present invention is to provide a low-cost alternative to typical fluorescence microscopy configurations. A feature of the device is the illumination of an entire sample and at an angle, or by some other means, which precludes exposure of the objective to incident or reflected illuminating radiation. An advantage of the device is that exposure of the detector to reflected light is eliminated without the need for dichroic mirrors, confocal pinholes, or other means to shield the detector.

Yet another object of the present invention is to provide an efficient, compact, and portable fluorescence microscopy method. A feature of the invention is illuminating the sample with modular, low-energy (and therefore low-heat) LEDs that can be battery powered. Another feature of the invention is that the LED provides a swath of light that can be positioned to illuminate the entire sample at once. An advantage of the method is that the simultaneous illumination of the entire sample facilitates parallel analysis of the sample located in different regions of the field of view (FOV). Another advantage is the ability to substitute, or use simultaneously, different LEDs so as to either serially- or simultaneously-illuminate the same sample with different radiation.

Briefly, the invention provides for a device for fluorescence microscopy comprising a means for illuminating a sample undergoing microscopic examination by juxtaposing a appropriately filtered light from one or more light emitting diode to the sample so as to irradiate the sample, a means for filtering and detecting radiation fluorescing from the sample and visually monitoring or permanently imaging the radiation from the sample, and a means for preventing said detecting means from detecting non-fluorescing radiation from the sample or fluorescent radiation that does not originate from the sample.

The invention also provides a fluorescence microscope comprising a means for supporting a sample; an objective lens opposing said support means; a means for simultaneously illuminating all points of the sample with incident radiation; and a means for preventing reflected radiation and the incident radiation from passing through said lens.

The invention also provides for a method for illuminating a microscopic construct, the method comprising providing an illumination detector capable of simultaneously monitoring all points of the construct; and simultaneously directing illuminating radiation to all regions of the construct and in a manner that prevents exposure of the detector to the illuminating radiation.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects and advantages of the present invention will become readily apparent upon consideration of the following detailed description and attached drawings, wherein:

FIG. 1 is a schematic depiction of a typical state-of-the-art confocal fluorescence microscope configuration;

FIG. 2 is a schematic depiction of an exemplary fluorescence microscope to provide dark-field epi-illumination using LEDs in combination with a ring mirror, in accordance with features of the present invention;

FIG. 3 is a schematic depiction of another fluorescence microscope but without a ring mirror, in accordance with features of the present invention;

FIG. 4a is a schematic depiction of a means for providing sample illumination using a fiber optic cable, in accordance with features of the present invention;

FIG. 4b is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables, in accordance with features of the present invention;

FIG. 4c is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables embedded in the housing of the objective, in accordance with features of the present invention;

FIG. 5 is a schematic depiction of illuminating microscopic samples from a non-perpendicular transmitted angle, in accordance with features of the present invention;

FIG. 6 is a perspective view of an LED-objective construct, in accordance with features of the present invention;

FIG. 7 is a cross sectional view of LEDs embedded into the housing for an objective, in accordance with features of the present invention;

FIG. 8 is a cross sectional view of an LED embedded into an objective, in accordance with features of the present invention;

FIG. 9 is a schematic view of a sample illumination configuration utilizing two parabolic mirrors, in accordance with features of the present invention; and

FIG. 10 is a schematic view of a sample illumination configuration utilizing two parabolic mirrors and a fiber optic cable, in accordance with features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A low-cost, highly optimized fluorescence microscope configuration is provided. Salient features of the invented device include the minimization of exposure of detectors to the excitation light (for example, reflected light) due to the off-specular-reflection angle placement of illuminating light emitting diodes. As such, the invented configuration resembles conventional epi-fluorescent microscope illumination wherein reflected light is directed away from the detector via a dichroic mirror.

The LEDs may be located inside the microscope, between the objective lens and the image detector, one possible configuration being a ring of LEDs coaxial with the microscope, the LEDs themselves being surrounded by an annular mirror that focuses the LED light onto the sample. Or the LED's light may be transmitted to the sample by means of optical fibers terminating in the region between the objective lens and the detector.

Also, one may have the LEDs encased in a container the inner walls of which are coated with a substrate that would reflect the LEDs' radiation onto the sample. Several other variations of LED placement and of means to reflect the LED light onto the sample can also be effected. All of these configurations minimize specular reflection from the sample of the fluorescing radiation into the microscope eyepiece and detector as well as direct incidence of the fluorescing radiation into the microscope eyepiece and detector. This eliminates signal-masking background that obscures the effect to be observed.

Furthermore, provision can be made for easy substitution of LEDs so that by using LEDs emitting light of different frequencies, one may excite different sample

resonances or different tag molecules and thus observe different features of the sample. Similarly, provision can be made for the interchange of different types of lens or mirror objectives or combinations thereof.

The LEDs small size, high power output, low heat output, high quantum yield, and their interchangeability allows one to design devices that cannot be built with other light sources. The use of LEDs results in fluorescence microscopes of smaller dimensions, simpler construction, better signal to noise ratio and higher sample illumination, $3mW/mm^2$ as compared to $1mW/mm^2$ with other light sources.

An exemplary configuration of the invented device is depicted in FIG. 2. Briefly, light from a light emitting diode 52 impinges upon a lens 54. The lens 54 transforms the light into a parallel beam 56. The beam 56 is directed to a ring mirror 58 which serves to redirect or bend the beam into a sheath 60. Generally cylindrical in form, the sheath 60 defines longitudinally varying inside surfaces 62 comprised of reflective material so as to facilitate rerouting of the light beam to a sample 64.

To enhance illumination of the sample, a portion of the reflective surface is formed into a frusto-conical configuration region 66 which converges toward the sample area. Alternatively, instead of a frusto-conical configuration, the sheath defines a parabolic section. As with the frusto-conical section described supra, the parabolic section defines inwardly facing surfaces converging toward the sample.

Generally, the sheath is configured to direct light to the sample so as to prevent any light reflecting off of the sample from being read by the detector 68. For example, the reflective surface of the sheath is configured so that light incident upon the reflective surface is directed at an angle toward the sample so as to prevent reflection of un-utilized excitation light into the field of view of the detector. Thus, only light emanating from the sample as a result of fluorescence of the sample is detected.

Utilizing the sheath also eliminates the state-of-the art requirement of illuminating the sample through an objective 63. As such, no optics fluorescence occurs and therefore a further decrease in background radiation is realized. Increased sensitivity and contrast results from this invented arrangement. This embodiment has become practical now that high output LEDs have become available.

The fluorescence emanating from the illuminated sample 64 is observed via an

eye piece 68 or recorded via a detector, such as photographic film, CCD camera, etc.. The light beam 56 impinges on the sample 64 for a time sufficient to induce the excitation of fluorochromes located in the sample

It may be noted that the embodiment illustrated in FIG. 2 may be simplified by omitting the sheaf 60 and by using a plane partially silvered or dichroic disk mirror instead of the ring mirror 58.

Instead of an LED situated outside of the sheath 60, one or a plurality of LEDs 52 are optionally situated inside the sheath, as depicted in FIG. 3. This configuration obviates the need for a ring mirror or a lens.

In either configuration, the reflective surface is configured to direct light to the sample 64 at an angle α so as to prevent any of the directed light, or any reflecting portion of the directed light to be read by a fluorescence-recording or -detecting means 68. Generally, an impinging light beam or reflected light beam which does not travel in parallel with the focal path of the microscope indicates that the LED and reflective surfaces are suitably oriented, relative to each other. A typical configuration therefore is side illumination of the sample, i.e, where an angularly and medially-directed illumination light is applied to the sample from a point lateral from the longitudinal axis of the microscope. Such a configuration obviates the need for dichroic mirrors, confocal pinholes or other filtering devices.

Depending on the configuration of the light emitting diode, and to provide even illumination to all fluorochrome-containing entities in the sample, the light radiation from the LED may first be directed to an optofiber system comprising an axial optical fiber 70 as depicted in FIG. 4a or a plurality of optical fibers 71 as depicted in FIG. 4b. Such optofibers disperse the light from an LED or from several LEDs, thereby providing more homogenous illumination of the sample 64. FIG. 4c is a schematic depiction of a means for providing uniform sample illumination using fiber optic cables embedded in a housing 61 of the objective 63.

Suitable optical fibers are available from a myriad of commercial suppliers, including Optical Cable Corporation of Roanoke, Virginia, USA, and Litkarino, of Moscow, Russia.

FIG. 5 depicts the utilization of LEDs in a dark-field configuration. Generally,

this configuration provides impinging radiation 57 at a non-perpendicular angle to the sample. The sample is illuminated in a dark field wherein the transmitted light beams impinge upon the sample at a point lateral from the midline 65 of the field of view.

That the impinging radiation contacts the sample 64 at an angle not perpendicular to the plane 72 upon which the sample rests is achieved via a cardioid, parabolic, or dark-centered condenser 73 juxtaposed intermediate the LED 52 and the sample 64. As such, the LED and the condenser are located distal from the detector relative to the sample 64 and below the plane 72 of the sample.

The arrangement depicted in FIG. 5 is particularly suited for viewing transparent samples mounted on transparent supports. This arrangement has the advantage that the exciting radiation does not impinge on the objective 63 and thus does not contribute to the background. Best results are obtained when the excitation beam is formed with the help of a parabolic condenser positioned between the LED 52 and the sample 64.

The inventors have utilized the invented fluorescence microscopy configuration to illuminate tagged moieties residing in nearly microscopic acrylamide substrates (0.1 x 0.1 x 0.02 mm). In such scenarios, wherein as little as 5×10^5 molecules of Texas red may be detected with average excitation intensities of between 0.5 and 10 mW/mm^2. Therefore, LEDs suitable for use in the instant invention should have light intensities ranging from approximately 0.5 to 50 milliwatts per square millimeter, depending on the spectral region targeted. Average exposure time is less than or equal to one minute. More typically, exposure times range from approximately 0.5 to 30 seconds.

LED Detail

A myriad of types of light emitting diodes lasers are utilized in the invented device. A suitable laser must emit a radiation suitable to induce fluorescence from typical chromophores used in tagging processes. Typical chromophores include, but are not limited to, Texas Red, Cy dyes, Naphthofluorescein, Fluorescein, BODIPY dyes and others. Table 1 shows the various types of fluorochromes that can be utilized with various LEDs currently available. A myriad of LED suppliers exist, including, but not limited to Agilent Technologies, Palo Alto, CA, Arachs Tech of

Hsinchu, Taiwan, Coombs Associates, Inc., of Arlington Heights, IL, Hewlett Packard, and Toshiba.

Table 1.

Source	LED Emitting λ	Labels used	Label's abs. max.
HPWL-BD01 Hewlett-Packard	629	CY 5	650 nm
		BODIPY 650	634 nm
LEAAA-100YE Arachs Technology	600	Naphthofluorescein	580-620 nm
		Texas Red	594 nm
LEAAA-100GN Arachs Technology	525	CY3	560 nm
		Lissamine	575 nm
		BODIPY TMR	545 nm
		Alexa	555 nm
LEAAA-100 BL Arachs Technology	470	Fluorescein	495 nm
MCDPG-3X0S Coombs Assoc.	450	Porphyrins	650 nm

Example

Sixteen blue-light LEDs 52 were installed along the periphery of a Zeiss objective 20 x 0.5". No additional lenses were utilized, inasmuch as the "effective illumination" area of each of the LEDs were approximately 10 mm in diameter. A perspective view of the LED-illuminated objective, is depicted in FIG. 6. Also depicted in FIG. 6 is a power-source 75 removably attached to a region of the objective's housing 76. The power source is provided for energizing the LEDs. For the sake of convenience, the LEDs typically are removably, and circumferentially arranged along the periphery of the objective's housing 76. The LEDs can be removably attached in a myriad of ways, including but not limited to a hook-and pile arrangement (e.g. VELCRO®), and a male-female configuration, whereby clips (not shown) arranged along the periphery are adapted to receive the LED's in a proper configuration for illuminating a sample juxtaposed to the objective. In this manner, LEDs can be substituted and the same objective can be utilized for various wavelength experiments.

Alternatively, as shown in FIG 7, the LEDs can be permanently affixed to the

objective housing with adhesive, mechanical fasteners, or integrally molded to the material forming said housing. This scheme allows design and use of specialized objectives which can be incorporated into an ordinary microscope and transforming it into a fluorescence microscope. The fluorescent lighting may be used instead of or in conjunction with ordinary microscope lights.

Upon energizing the LEDs, a total illuminated area of approximately 10 mm in diameter resulted, which was larger than the objective's field of view. As such, any illuminated area at least as large as the objective's field of view is suitable.

The intensity of illumination inside the field of view of the 20 x 0.5 objective was approximately 3 mW/sq. mm. This intensity is used to excite FITC, which is an activated derivative of fluorescein. With a standard emission filter for FITC and utilizing Polaroid® ISO-3000 film as the detector recording medium, exposure time for tubercle bacilli immersed with fluorescein, and washed, was approximately 2-3 seconds. This exposure time is comparable with those provided by commercially-available fluorescence microscopes.

A variation of the LED-objective configuration depicted in FIG. 6 is illustrated in FIG. 8. In FIG. 8, the LED 52, or a plurality of LEDs contact a region 67 of the objective 63 which defines the field of view. In the depicted embodiment, the LED is partially imbedded into the surface of the lens which is proximal to the sample 64 and distal from the detector (not shown in FIG. 7). In the embedded configuration, the LED is positioned at a distance "D" within 300 μm of the sample so as to obviate the need for additional focusing means.

To prevent background radiation from reaching the detector, the region of the lens which receives the LED is treated with an opaque material 69 or otherwise "blacked out" to the detector. Furthermore, inasmuch as the electrical conductors (for example, wires) energizing the LED are not located in the object plane, the conductors are not visible in the field of view. As such, their presence will not obstruct the field of view but may instead slightly decrease the intensity of illumination or spatial resolution. This scheme is most promising but it requires adapting present-day objectives.

FIG. 9 depicts an illumination configuration utilizing two parabolic mirrors. First, the sample, 64 is directly illuminated by a light source 52. The light source directs energizing radiation away from the detector 68. This radiation from the light source

first passes through a lens 54.

Fluorescing radiation 55 emanating from the sample impinges upon a first parabolic reflective surface 80 and is then redirected to a second parabolic surface 82. The twice reflected radiation 64 is finally observed through an eye piece 68 or other detection means.

In the embodiment depicted in FIG. 9, the first parabolic mirror 80 is concentrically arranged about the longitudinal axis α of the microscope with the surface of the mirror deviating medially, or inwardly toward the axis. The parabolic configuration of the first reflective surface depends downwardly toward a plane 86 supporting the sample.

The second reflective surface 82 also is of a parabolic-shaped substrate concentrically arranged relative to the longitudinal axis α to converge medially therewith. However, this second surface is directed away from the sample so as to oppose the first reflective surface. As such, the second surface is directed upwardly to receive first reflected radiation 83 from the first reflective surface.

The first and second reflective surfaces are optimized in albedo and configuration to maximize capture of fluorescing radiation emanating from the sample.

An advantage of the dual reflectance configuration of FIG. 9 is the complete isolation of the detector 68 from non-fluorescing radiation emanating from the light source 52. To assure complete isolation of the detector 68 to unutilized energizing radiation, a backstop 88 of the light source, a backstop 90 of the first reflective surface 80 and a backstop 92 of the second reflective surface 82 are configured to be opaque to the energizing radiation utilized in the device depicted in FIG. 9. This scheme is made possible by the present availability of small-size LEDs together with large size mirror objectives.

As a further alteration to FIG. 9, the light source 52 need not be incorporated into the dual mirror configuration. Instead, as shown in FIG. 10, one or a plurality of optical fibers 70 can illuminate the sample 64 by directing light to the sample from an LED 52 or plurality of LEDs not embodied by the device. As shown in FIG. 4b, significant increase in the illumination intensity may be achieved by having the fluorescence radiation be brought to the sample by optical cables and by placing the end of the cables near the sample.

While the invention has been described with reference to details of the illustrated embodiments, these details are not intended to limit the scope of the invention as defined in the appended claims. For example, the utilization of CCD cameras in the invented device would obviate the need for changing the objectives or other magnification means, inasmuch as CCD cameras confer the ability to change the range of magnification digitally.

CLAIMS

The embodiment of the invention in which an exclusive property or privilege is claimed is defined as follows:

1. A device for illuminating a sample undergoing microscopic examination, the device comprising:

a) a means for detecting radiation;

b) a light emitting diode juxtaposed to the sample so as to irradiate the sample; and

c) and means to prevent said detecting means from detecting radiation not emanating from the sample.

2. The device as recited in claim 1 wherein the light emitting diode is embedded in an objective lens.

3. The device as recited in claim 1 wherein radiation from said light emitting diode impinges upon the sample at an angle that is not perpendicular to a plane upon which the sample rests.

4. The device as recited in claim 1 wherein radiation from said light emitting diode impinges directly upon the sample.

5. The device as recited in claim 1 wherein radiation from said light emitting diode is reflected onto the sample.

6. A fluorescence microscope comprising:

a) a means adapted to support a sample;

b) an objective lens opposing said support means;

c) a means for simultaneously scrutinizing all points of the support means; and

d) means for illuminating said support means with radiation which does not first pass through said lens.

7. The microscope as recited in claim 6 wherein the illuminating means is one or more light emitting diodes (LEDs).

8. The microscope as recited in claim 6 wherein the scrutinizing means is photographic film.

9. The microscope as recited in claim 6 wherein the radiation contacts the support means from a point lateral to a line defined by the lens and an opposing point on the support means.

10. The microscope as recited in claim 7 wherein the LEDs rotate around their longitudinal axis.

11. The microscope as recited in claim 6 wherein the radiation is filtered before impinging on the sample.

12. The microscope as recited in claim 6 wherein the radiation is filtered before impinging on the scrutinizing means.

13. The microscope as recited in claim 6 wherein the illuminating means comprises an LED that is mobile relative to the support means.

14. The microscope as recited in claim 6 wherein the sample support means are movable.

15. The microscope as recited in claim 6 wherein the illuminating means is one or more LEDs that are stationary relative to the support means.

16. The microscope as recited in claim 7 further comprising a ring mirror positioned between the objective lens and the scrutinizing means, said mirror adapted to reflect light from the LEDs onto the sample.

17. The microscope as recited in claim 7 wherein said LEDs are embedded in the objective lens.

18. The microscope as recited in claim 16 wherein the ends of the fiber optic cables are located between the objective lens and the scrutinizing means.

19. The microscope as recited in claim 7 further comprising a sheath encircling the objective lens, said sheath adapted to reflect light from the LEDs onto the sample.

20. The microscope as recited in claim 19 wherein the LEDs are positioned between said sheath and said objective lens.

21. The microscope as recited in claim 6 wherein the illuminating means comprise fiber optic cables

22. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are located between the objective lens and the sample.

23. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are juxtaposed to the sample.

24. The microscope as recited in claim 21 wherein the ends of the fiber optic cables are embedded in the objective lens.

25. The microscope as recited in claim 6 wherein the sample support means are movable.

26. The microscope as recited in claim 7 wherein the LEDs are located intermediate said sample and said objective lens.

27. The microscope as recited in claim 7 wherein the LEDs are located around said objective lens.

28. The microscope as recited in claim 7 wherein the objective lens is replaced by an objective assembly comprising one or more mirrors.

29. The microscope as recited in claim 28 wherein the LEDS are positioned between the objective assembly and the sample.

30. The microscope as recited in claim 28 wherein the sample is illuminated by fiber optic cables located between the sample and the objective assembly.

31. The microscope as recited in claim 7 wherein the LEDS all emit light are positioned between the objective assembly and the sample.

32. The microscope as recited in claim 28 wherein the LEDS each have a specific light spectrum.

33. The microscope as recited in claim 32 wherein the LEDS have the same specific light spectrum.

34. The microscope as recited in claim 32 wherein one or more of the LEDS

have a different specific light spectrum from one or more LEDs.

35 The microscope as recited in claim 7 wherein one or more of the LEDS may be rotated around an axis of the microscope.

36 The microscope as recited in claim 7 wherein one or more of the LEDS are adapted to be quickly replaced.

37. A method for illuminating a microscopic construct, the method comprising:

a) providing an illumination detector capable of simultaneously monitoring all points of the construct; and

b) directing illuminating radiation to the construct in a manner that prevents exposure of the detector to the illuminating radiation.

38. The method as recited in claim 37 wherein the source of illuminating radiation is an LED.

39. The method as recited in claim 37 wherein the illuminating radiation is derived from a plurality of LEDs.

40. The method as recited in claim 39 wherein each of the LEDs have a different wavelength and the beams impinge on the construct serially.

41. The method as recited in claim 39 wherein each of the LEDs have the same wavelength and impinge on the construct simultaneously.

42. The method as recited in claim 38 wherein the LED is in motion relative to the construct.

43. The method as recited in claim 38 wherein the LED is rotating about its longitudinal axis.

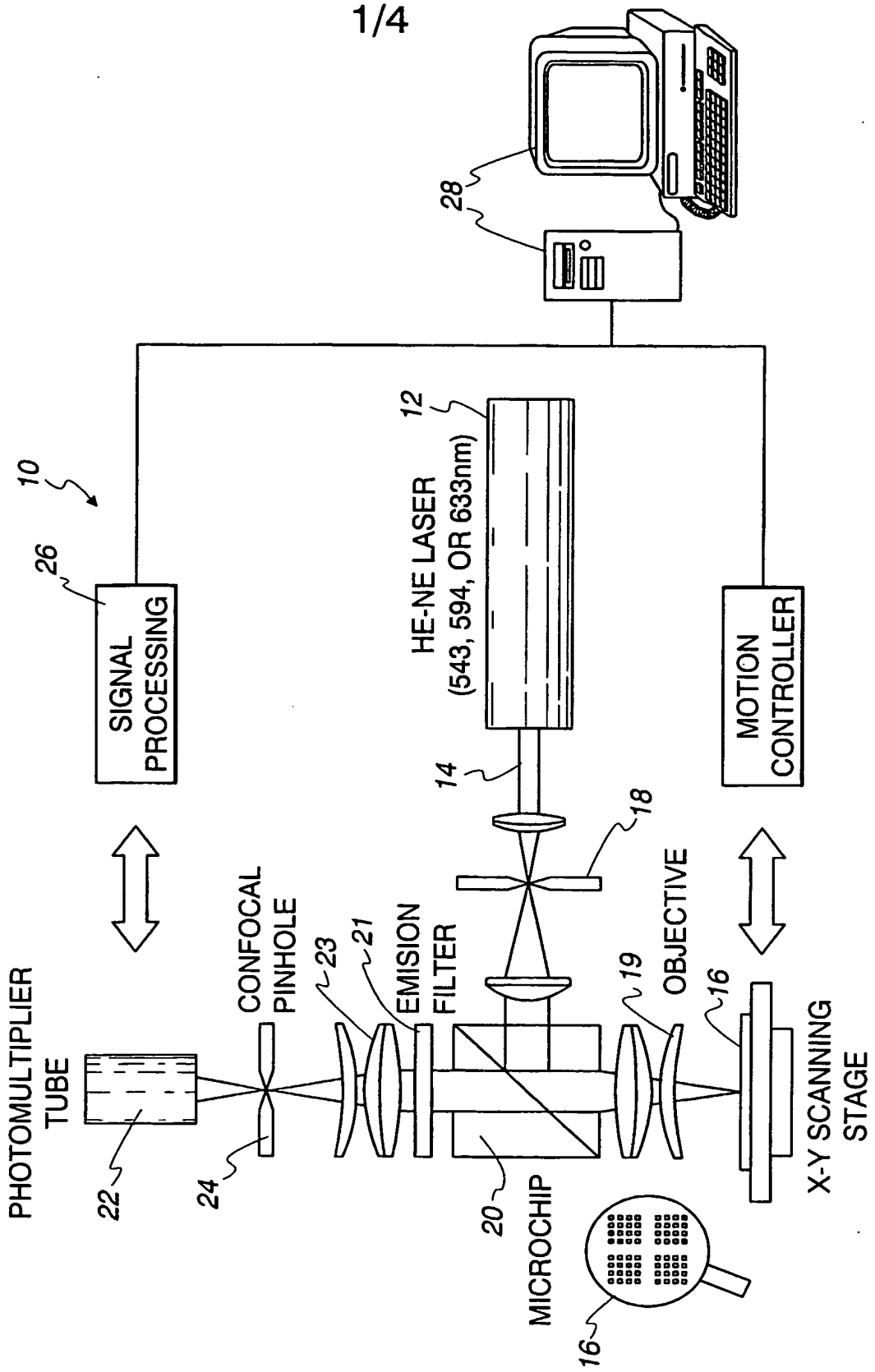

28
10
26
SIGNAL PROCESSING
12
HE-NE LASER (543, 594, OR 633nm)
MOTION CONTROLLER
14
18
PHOTOMULTIPLIER TUBE
CONFOCAL PINHOLE
23
21
EMISION FILTER
19
OBJECTIVE
16
X-Y SCANNING STAGE
22
24
20
MICROCHIP
16

Fig. 1

Fig. 2

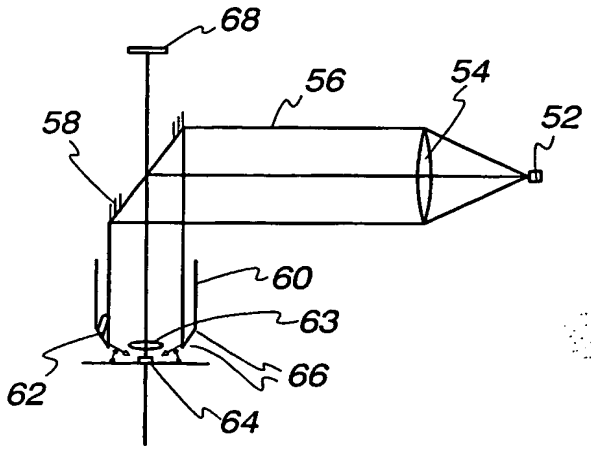


Fig. 3

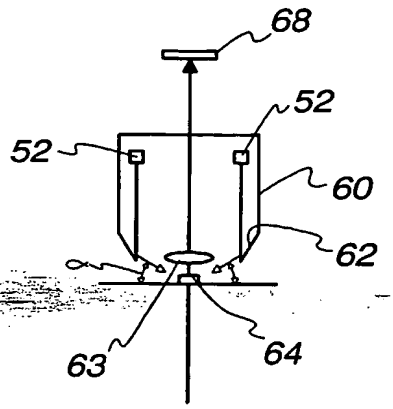


Fig. 5

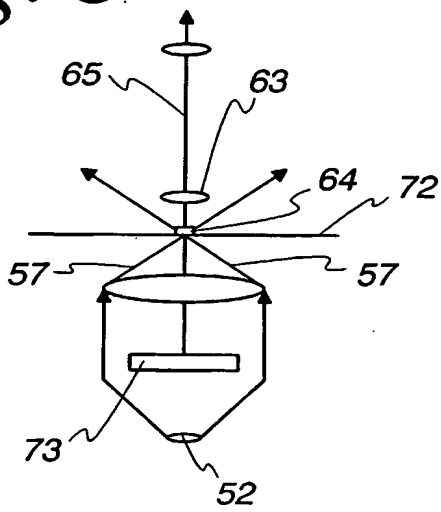


Fig. 6

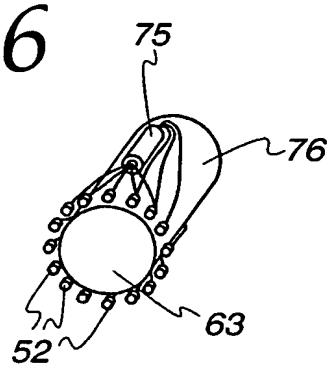


Fig. 7

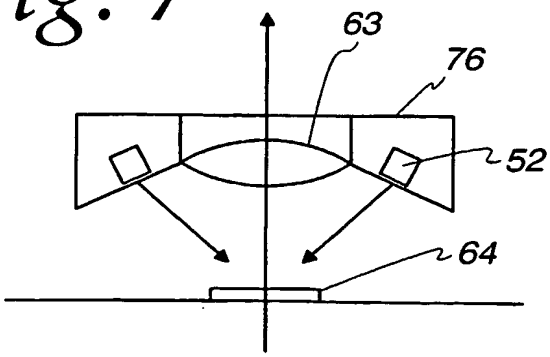


Fig. 8

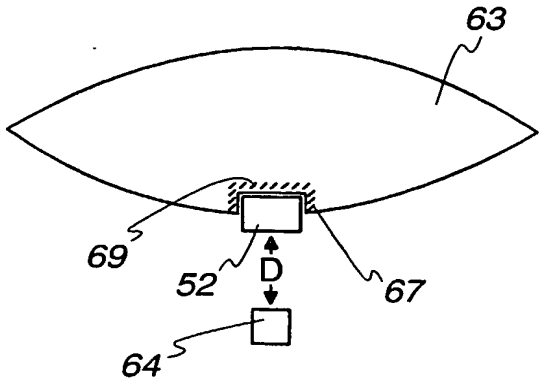


Fig. 4a

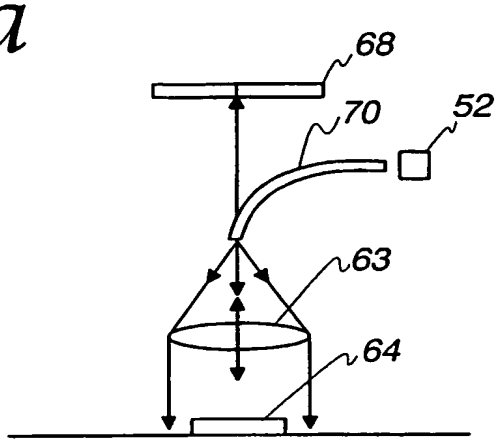


Fig. 4b

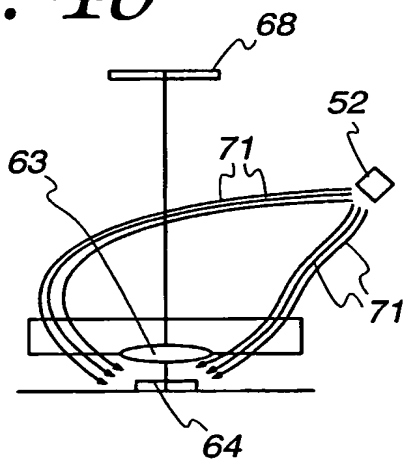


Fig. 4c

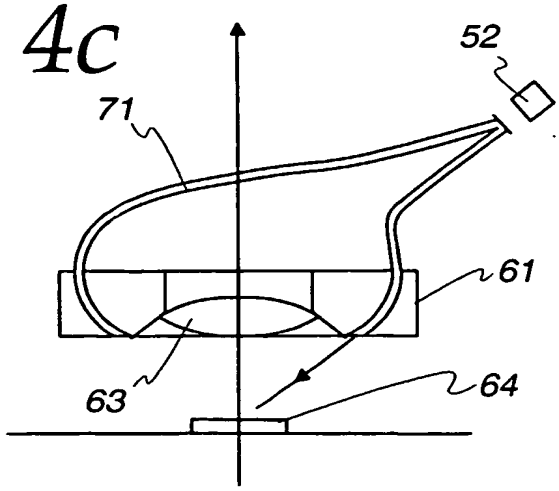


Fig. 9

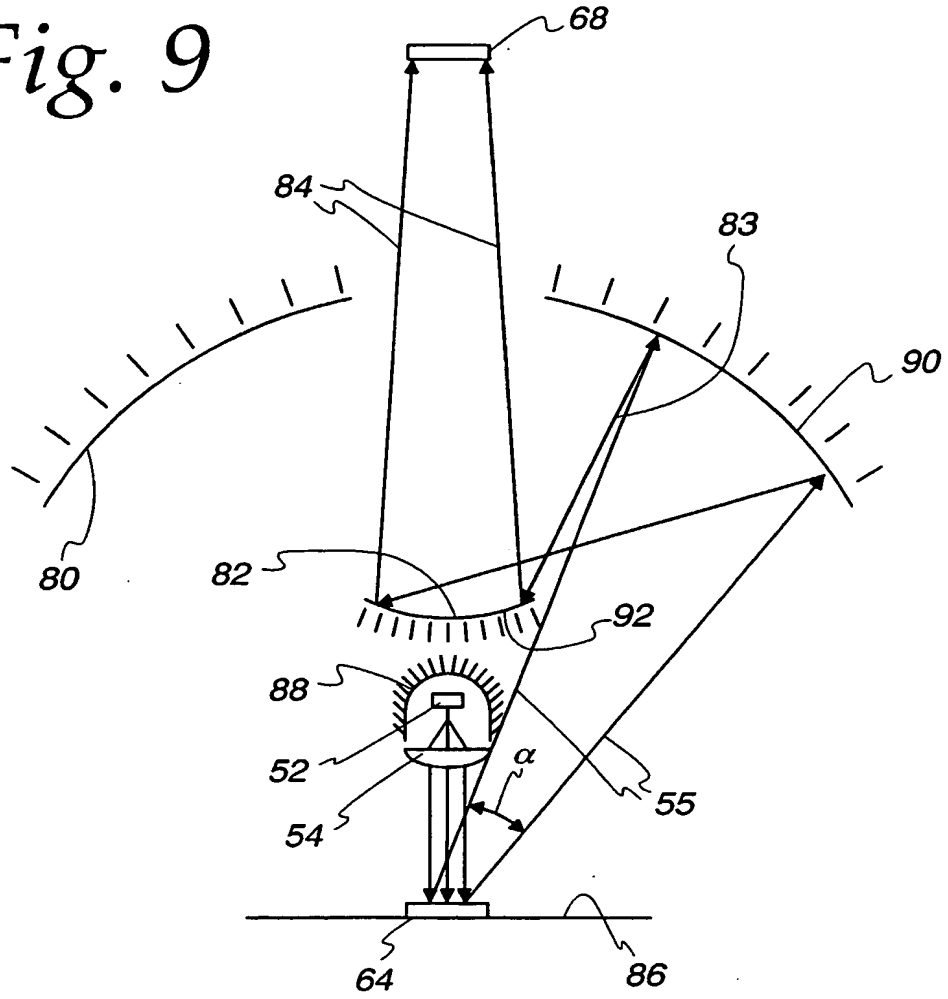
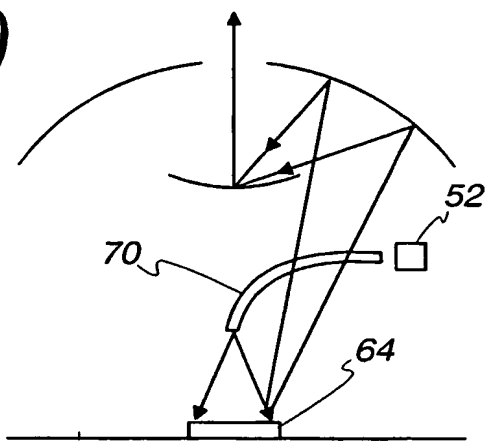


Fig. 10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05107

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/64
US CL :356/417, 317, 318; 250/458.1, 461.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/417, 317, 318; 250/458.1, 461.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST
search terms: microscope, fluorescence, light emitting diode, scanning, LED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 3,705,755 A (BAER) 12 DECEMBER 1972 (12-12-72), see entire document.	1, 5, 6, 7, 15, 32 --- 28, 33, 34, 37, 38, 39
X --- Y	US 5,646,411 A (KAIN et al.) 08 JULY 1997 (08-07-97), see entire document.	1, 3-7, 14-15, 25-26, 31, 33-35, 36-40 --- 9-13, 16, 21-3, 42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 APRIL 2001	Date of mailing of the international search report 08 MAY 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ZANDRA V. SMITH Telephone No. (703) 305-7776

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/05107

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 6,008,892 A (KAIN et al.) 28 DECEMBER 1999 (28-12/99), see entire document.	1, 3-8, 14-15, 25-26, 31, 33-34, 36-40 ---------- 9-13, 16, 21-23, 42
X,P ---- Y	US 6,154,282 A (LILGE et al.) 28 NOVEMBER 2000 (28-11-00), see entire document.	1, 4, 5 ---------- 6-7, 9, 14-15, 25, 28, 32-34, 37-39